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CULTIVATION of the SAPOGENIN-BEARING *DIOSCOREA* SPECIES

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CONTENTS

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CULTIVATION of the SAPOGENIN-BEARING *DIOSCOREA* SPECIES

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SUMMARY

Sapogenin-bearing *Dioscorea* species yield the most widely used precursors for synthesis of steroidal drugs. The species *D. composita* Hemsl. and *D. floribunda* Mart. and Gal. are especially suitable for cultivation. Suitable agronomic techniques have been developed chiefly under Puerto Rican conditions, but experiments have shown that the species may also be grown in southern Florida.

Plants grow well from seeds. Success with vegetative propagation from leaf or stem cuttings or tuber pieces varies, depending upon species and season.

Propagation from seeds is considered the most reliable method; however, although more expensive and less reliable, asexual propagation may be used profitably under some circumstances. Seedling plants grow slowly and direct field seeding has not been successful. The most satisfactory method yet developed is to grow plants in seedbeds until they are from 4 to 6 months old, depending upon the species, and then transfer them to field plantings.

Dioscorea species tolerate many soil and climatic conditions. However, results are best with loamy well-drained soils, in locations of long rainy seasons. Although the plants are drought tolerant, supplemental irrigation during dry weather is advisable, especially immediately after planting. Soil type and depth will affect the shape of the tubers and the ease of harvest.

Plants require support for satisfactory growth. Post-and-wire and stake supports are satisfactory, but expensive. Various living plants have been tested as supports, but none has been satisfactory.

Although mature plants are vigorous and aggressive, young plants are not. Consequently, weed competition with new plantings is severe during the first season of growth. Conventional cultivation techniques to control weeds often must be supplemented with handweeding.

Aphids and spider mites sometimes attack the vines. No specific recommendations have been made to control these pests, but standard practices such as those used with other crops have been successful. A virus disease that occasionally appears can best be controlled by prompt eradication of infected plants and treatment to control insects that may act as vectors.

Tubers may be harvested when they are 3 or 4 years old. Harvesting in the dry season has been most practical in Puerto Rico. Vines and supports must be removed beforehand, and tubers plowed up and separated from the soil. Tubers of *D. floribunda* can be harvested more easily than those of *D. composita.* Harvested tubers must be washed and at least partly dried if they are not to be processed immediately.

A market has not yet been established for tubers of sapogenin-bearing *Dioscorea* species, and thus prospective agriculture operations will have to be closely related to the development of processing facilities. However, with the increasing number of prospective uses of steroidal drugs, the future of cultivated dioscoreas looks promising.

INTRODUCTION

Following the demonstration in 1948 of the therapeutic properties of steroid hormones in the treatment of arthritic diseases, widespread clinical use caused an acute shortage of these hormones. The supply as a byproduct of animal slaughter was unable to meet the sharply increased demand, and an intense search commenced for suitable plant sources of steroid precursors. Then development of clinical techniques to suppress ovulation with steroid drugs produced a further dramatic increase of interest in these compounds. In 1955 D. S. Correll and his coworkers described the manner in which interest gradually narrowed to the genus *Dioscorea* as the most promising plant source of steroid precursors. Since then *Dioscorea* species have been collected from all parts of the world and assayed for steroidal sapogenins.

Since 1950, interest in *Dioscorea* species has developed in many different countries. Wild tubers are being commercially harvested for their sapogenins in Mexico, India, China, Africa, and Europe. A large number of sapogenin-bearing species have been found. The most promising of these and their geographic source are listed in table 1.

TABLE 1.—*Principal sapogenin-bearing* Dioscorea *species and their geographic sources*

Species	Geographic source
D. balcanica Kosanin	Europe.
D. belizensis Lundell	Central America.
D. composita Hemsl.	Mexico, Guatemala.
D. deltoidea Wall.	India.
D. floribunda Mart. & Gal.	Mexico, Central America.
D. friedrichsthalii R. Knuth	Central America.
D. glauca Muhl.	United States.
D. hondurensis R. Knuth	Mexico, Central America.
D. mexicana Guill.	Mexico, Central America.
D. prazeri Prain & Burk.	India.
D. spiculiflora Hemsl.	Mexico, Central America.
D. sylvatica Ecklon	Southern Africa.
D. villosa L.	United States.

In 1950 the U.S. Department of Agriculture began to study the agronomic potential of several sapogenin-bearing *Dioscorea* species. The Department has emphasized the development of the *Dioscorea* species as a cultivated crop. Studies have been made both in the continental United States and in Puerto Rico. Intensive study at the Federal Experiment Station, Mayaguez, has demonstrated the feasibility of cultivating these plants for steroid production. Some commercial plantings are now established in Puerto Rico.

This bulletin describes and discusses techniques developed for growing the two *Dioscorea* species (*D. composita* and *D. floribunda*) that are apparently best suited as cultivated plant sources of steroid precursors. Most of the work has been documented in scientific publications. A complete list of these begins on page 18.

SPECIES AND VARIETIES

Cultural practices must conform to differences among species. Prominent characteristics of the four most suitable sapogenin-bearing *Dioscorea* species are as follows:

D. composita Hemsl. Usually this species propagates poorly from stem cuttings and only fairly well from tuber pieces. Seeds germinate slowly until about 1 month after harvest, then require about 3 to 4 weeks in petri dishes or 2 to 6 weeks in seedbeds. Vines establish within 2 months after germination, but plants are not strong enough for field planting until they are 4 to 5 months old. Vines are robust and long; they often cross between supports and thus shade weeds. Tubers are white, large (5 kilograms), long (1.5 meters), and deep (0.5 meter) (fig. 1); they are susceptible to rot. Diosgenin percentage is fair (4 to 6 percent). In Puerto Rico this species is affected less by virus diseases than other species studied. During the dry season, vines remain green.

D. floribunda Mart. & Gal. Usually this species propagates well from stem cuttings or tuber pieces.

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FIGURE 1.—Mature tubers of, *left*, *D. floribunda* and, *right*, *D. composita*.

Seeds germinate well in 3 to 4 weeks in petri dishes or 2 to 6 weeks in seedbeds. Seedlings are slow to become established but are vigorous later. Plants are strong enough for field planting when they are 5 to 6 months old. Tubers are yellow, small (3 kilograms), more compact than *D. composita* (0.8 meter), and shallow (seldom over 0.3 meter) (fig. 1); branches are thick. Diosgenin percentage is good (6 to 8 percent). In Puerto Rico this species is very susceptible to virus diseases. During the dry season, the plants are inclined to dormancy and vine dieback.

D. friedrichsthalii Knuth. This species is less well known than either *D. composita* or *D. floribunda*. Seeds germinate rapidly, and plants are strong enough for field planting when they are 4 months old. Vines are intermediate to low in vigor. Tubers are white, intermediate in size (4 kilograms), and compact (0.5 meter). Sapogenin percentage is low (3 percent). In Puerto Rico this species is extremely susceptible to virus diseases. During the dry season, the plants are inclined to dormancy and vine dieback.

D. spiculiflora Hemsl. This species propagates exceptionally well from juvenile rosettes, but not from tuber pieces. Seeds require several weeks for germination. Plants seldom produce long stems in their first year. This species tolerates acid soil less satisfactorily than the other species. Vines of established plants are less vigorous than those of *D. composita* and *D. floribunda*. Tubers are white, small (2 kilograms), compact (0.5 meter or less), and shallow (0.25 meter). Sapogenin percentage is very high (8 to 14 percent). In Puerto Rico this species is quite resistant to virus diseases. Dormancy is pronounced in the dry season or under short daylengths, regardless of moisture availability. Vine dieback occurs regularly in the winter season. Because of its small tuber size, *D. spiculiflora* does not yield as well as *D. composita* and *D. floribunda*. Also, *D. spiculiflora* contains a mixture of sapogenins that cannot be readily separated commercially. Therefore, despite its high total sapogenin content and its resistance to virus diseases, *D. spiculiflora* is not at this time (1968) considered suitable for commercial planting.

METHODS OF PROPAGATION

Propagation From Seeds

The seeds of all sapogenin-bearing *Dioscorea* species discussed here are about 1 millimeter thick and 1.5 centimeters in diameter (fig. 2). The bulk of the seed is a wide, membranous wing that can be removed without affecting germination. The embryo and endosperm are 3 to 5 millimeters in diameter. Seeds can be stored 3 or more years in a household refrigerator if kept thoroughly dry with a desiccant such as calcium chloride. Small quantities of seeds can be germinated on moist filter paper in petri dishes. Large quantities are best germinated in seedbeds. Seeds germinate best in a light, well-drained medium, such as a loamy soil, kept moist but not wet. The seedbed should be protected from drying by light shade. For maximum production of plants from limited seed supplies, seeds can be evenly spaced from 1 to 1½ inches apart by using a pegboard to mark holes. If a large supply of seeds is available, they can be broadcast over the prepared surface of the seedbed. Planting depth should be ¼ to ½ inch. Covering the seeds with a mixture of sand and shredded peat moss helps maintain the physical conditions necessary for germination. The beds must be watered with care, lightly and frequently, to avoid disrupting the seed.

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FIGURE 2.—Seeds of *Dioscorea* species: *Top, left, D. composita* and, *right, D. floribunda; bottom, left, D. friedrichsthalii* and, *right, D. spiculiflora.*

Germination usually begins in 3 weeks, with the emergence of a single-leaf shoot (fig. 3). Several shoots appear over a 2- to 3-week interval and form a rosette of small leaves. Eventually one shoot will continue to lengthen as a vine. If the young vines do not find support quickly, they fall over and eventually die. Another shoot will usually follow, but growth will be delayed if a support is not available. A simple technique to provide support is to place poultry wire horizontally 4 to 5 inches above the surface of the seedbed. The vines quickly reach the wire and build a tangled mass of foliage above. The wire can be bent to an undulating shape so that it touches the ground at intervals and supports itself, but a support of this type is more difficult to remove later. It is preferable to fasten the wire to the tops of stakes driven into the ground. Small rods or bamboo twigs have also been used as vine supports, but are considered less satisfactory than wire. If used, the rods or twigs should be spaced at least 4 per square foot so that all vines are within 6 inches of support. Each rod or twig may be covered by hundreds of tiny vines.

The young vines should be fertilized regularly for maximum growth. Detailed studies have not been made to indicate quantities or formulas most satisfactory for seedlings. Therefore, quantities or formulas commonly used for growing other plant species have been used for *Dioscorea* species also. Light (100 to 200 grams per square meter) but frequent (2- to 3-week intervals) applications of balanced mineral fertilizers have been successful.

In Puerto Rico, fungus leaf diseases have seldom created serious problems, and fungicidal treatments have usually not been required. However, severe fungus leaf diseases have been observed in other regions. Consequently, a commercial fungicide should be available to use if necessary.

The time of planting is important. Plants survive best and grow most rapidly during the long summer days of the rainy season. Seedbeds should be started in winter so that plants are ready to be moved to the field as soon as summer rains occur regularly. The minimum period of growth in seed-

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FIGURE 3.—Seedling of *D. floribunda* showing origin of tuber from stem tissue.

beds is 4 months, but the plants will be more suitable for transplanting if grown for 6 to 8 months in seedbeds. Thus, plants started from seed in January would be ready for field planting from May to July. If the plants are set into the field too late in the summer, they may become dormant before making new growth, and new sprouts will not develop until the following spring. In this case it seems preferable to hold plants in the seedbeds through the months when they are dormant rather than plant them in the field very late in the summer when vigorous growth is unlikely.

Seedlings can be grown individually in small pots or plant bands with a light stick provided as a vine support. It is then possible to transplant the young plant intact, without pruning, and to avoid the retardation induced by pruning and bare-root planting. This procedure is recommended where maximum growth is desired from a small number of seedlings, but is not considered practical for large-scale production.

Propagation From Leaf or Stem Cuttings

In addition to propagation from seeds, dioscoreas may also be propagated by leaf or stem cuttings or by tuber pieces.

Leaf cuttings are leaves pulled or cut from the stem. The pulvinus at the base of the petiole must be retained intact. Stem cuttings are a single leaf attached to a part of the internode on either side. In *D. spiculiflora*, the individual stems of the juvenile rosette may be separated and repropagated so that a single seedling can be multiplied more or less indefinitely. Cuttings should be washed and treated with a mild fungicide before they are placed in the cutting bed. Excessive use of fungicide may be deleterious. In some experiments, rooting has been increased through use of a commercial hormone-type rooting agent.

Rooting beds must be well aerated and well drained. Media used for rooting are sand, gravel, peat moss, sphagnum moss, perlite, and vermiculite. Although we have preferred river gravel at the Federal Experiment Station at Mayaguez, P.R., we have no unequivocal evidence suggesting its superiority as a rooting medium. The watering method is probably as important as the rooting medium. Cuttings must be kept damp, and this is most easily done with an automatically controlled intermittent mist spray. Beds must be periodically dried and cleaned to avoid accumulations of algae and fungi. Fungi may destroy a bed of cuttings quickly if good sanitation is not practiced. Cuttings or rosettes are placed to root by burying the stem or petiole in the medium, with the leaf supported above the ground level with rods or chicken wire screen. Cuttings will root in full sun if protected against desiccation by intermittent mist, but light shade reduces water requirements and is considered desirable.

Rooting begins at the base of the pulvinus or in the axil of the leaf when new tuberous tissue develops. Roots and stems then grow from the small tuber. Some of the new tubers fail to form shoots as rapidly as desired. Preliminary experiments suggest that sprouting may be increased by placing such small tubers, with or without the parent leaf, in a closed container with vapor of 0.25 milliliter of ethylene chlorohydrin per liter of air space for 8 hours. Experimental results suggest that this concentration may be either useful or ineffective, but not harmful to germination. Higher concentrations of ethylene chlorohydrin have at times injured the tubers and reduced or delayed sprouting. The ethylene chlorohydrin treatment is most likely to be effective in the winter dry season (December to March) when the plants display some dormancy.

Two of the species studied in Puerto Rico, *D. floribunda* and *D. spiculiflora*, grow very little under short-day winter conditions. Maintaining long-day photoperiods by using supplementary lighting is recommended as a means of avoiding dormancy problems when propagating these species in winter.

Vines developing from the cuttings are a management problem in rooting beds. To prevent crowding, plants must be removed and transplanted regularly to pots, plant bands, or larger beds where they can grow larger and harden in preparation for field planting.

Propagation is most rapid from fully expanded but young leaves obtained from vigorously growing stems. This type of juvenile material is most readily available from the vigorous growth flushes that occur shortly after the rainy season begins. Propagation from cuttings becomes increasingly difficult later in the season as the plants approach flowering and dormancy in the fall.

In summary, propagation from cuttings or rosettes requires more facilities, is more costly, and is subject to more variables than propagation from seed. Propagation from cuttings or rosettes is best used to multiply superior clonal varieties or to preserve special plants.

Propagation From Tuber Pieces

Propagation from tuber pieces is accomplished by cutting the tubers into pieces weighing about 50 grams each. Three types of pieces can be distinguished: heads (stem ends), tips (terminal pieces), and intermediate sections. Heads produce new shoots within a few weeks after planting, indicating the presence in the head of preformed buds. Tips do not germinate well and should not be planted. Intermediate sections germinate in 3 to 6 months from new buds developed in the cambium of the upper surface of the tuber. During the relatively long period of this waiting for development of buds, the tuber pieces often decay. For this reason, tubers to be used for propagation should be dug with care, thoroughly washed, and treated with a fungicide recommended for tuberous propagules. The tuber pieces should be allowed to dry for several days before they are placed in the rooting bed. Diseased or seriously injured tubers should not be used for propagation.

Before the tuber pieces are placed in the germination medium, they may be treated with ethylene chlorohydrin as a possible means of stimulating bud sprouting. Data from experiments with ethylene chlorohydrin have been conflicting, and conclusive results are not available. Preliminary experiments suggest that sprout formation may be stimulated by placing the tuber pieces in a closed chamber with vapor of 0.06 to 0.12 milliliter of ethylene chlorohydrin per liter of air space for 8 hours. We believe this treatment is useful when plants are dormant, but not when normal germination is satisfactory. Experimental results suggest that this concentration may be either useful or ineffective, but not harmful to germination. However, higher concentrations of ethylene chlorohydrin have at times inhibited bud sprouting.

Tuber pieces can be planted directly in the field, but successful techniques for avoiding severe weed competition have not been developed. Consequently, it is advisable to hold tuber pieces in a moist, well-aerated rooting medium until shoot growth commences. The rooting medium should not contain more moisture than that necessary for root growth. Heating the rooting beds to 37° C. has increased rate of sprouting but has also encouraged tuber decay. Consequently, if a bed is heated, the tuber pieces should be watched carefully. If any of the tuber pieces begin to decay, the temperature should be lowered.

Seedling plants do not propagate uniformly. Tuber pieces from some sprout more readily than from others. We have not selected and maintained clones that propagate easily but for commercial planting with vegetative propagations this would be necessary. In all cases propagation from tuber pieces is more successful in spring and summer than in fall and winter.

To summarize, propagation from tuber pieces may be considered a suitable method to establish new plantings rapidly, especially those of selected clones. Although further experiments may improve techniques, the cost of establishing plantings by this method is likely to remain high. This cost is at least partly offset by the rapid growth of propagated plants and the consequent higher yields of fields propagated from tuber pieces.

PLANTING THE FIELD

Dioscorea plants (fig. 4) grow well in a wide variety of soils. In light, sandy soils heavy fertilization is necessary, and irrigation must be available to compensate for excessive drainage and lack of moisture during dry periods. Tubers in sandy soils tend to be small, but are easily harvested and cleaned. Extremely heavy clay soils restrict plant growth and impede later harvests. Best growth has been observed in well-drained loamy soils free from sticky clay. Such soils facilitate harvest, particularly of the deep-rooted species *D. composita.* Dioscoreas tolerate a fairly wide variation in soil pH, but very acid soils should be avoided, or limed before use, especially when *D. spiculiflora* plants are grown.

The soil and its preparation affect the morphology of the tuber. Tubers grow longer, thinner, and deeper in light soils than in heavy soils. A hard subsoil deflects the tubers and leads to horizontal growth. In some cultural experiments, trenches led to shallow, horizontal growth.

In some experiments, plants were set on raised beds. This planting system facilitates harvest since the tubers are more easily excavated from beds than from level ground. However, yields did not increase in raised beds. Also, yields did not increase when composted sugarcane filter press cake was added to the soil during preparation of the beds. These tests on raised beds indicate that on well-drained soils, soil preparation need be only sufficient plowing and harrowing to provide a loose bed approximately 8 inches deep. If poorly drained soils must be used, raised beds are recommended to increase soil aeration.

Field planting can be done by hand, but a mechanical transplanter will save time and labor. Vegetable-transplanting machines can be used successfully to plant *Dioscorea* species.

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FIGURE 4.—Plants of *Dioscorea* species: *Left*, well-established vines of *D. composita; right*, details of leaf and fruit of *D. composita*.

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Plants of *D. floribunda*, *D. friedrichsthalii*, and *D. spiculiflora* can be as close as 1 foot apart, whereas those of *D. composita* should be 1½ to 2 feet apart. Four-foot row spacing has been used in most experiments at the Federal Experiment Station at Mayaguez and is therefore recommended as the conventional procedure. However, the relationships between plant density and yield have not been studied thoroughly. Future investigations may reveal that higher plant densities may increase yields.

Crowns of the plants should be level with the top of the soil, but tuber pieces should be completely covered by 1 to 2 inches of loose soil. Plants should be "watered in" for best establishment. Nutrient starter solutions have not been tested, but these might prove useful if the soil is not fertile. Although *Dioscorea* species plants are somewhat drought tolerant, soil should not be allowed to become dry just after transplanting.

Vine supports should be provided as soon as plants begin to grow. Small bamboo twigs set very close to the plants can be used temporarily, but soon after planting a permanent staking system will be needed. A suitable vine support system is one of the major problems in cultivating *Dioscorea* species. Attempts to grow the plants without support have failed; the vines grow slowly and weed competition becomes severe. Intercropping *Dioscorea* with a living support has been tried, but in Puerto Rico all species tested as living supports have competed severely with the dioscoreas and reduced yields considerably. Nevertheless, finding a suitable living support could very effectively reduce production costs. Nonliving supports can be constructed from a variety of materials. The characteristics desirable in any support system, in addition to low cost, are sufficient height (5 to 6 feet), individual support for each vine, strength to carry hundreds of pounds of vine, and durability for 3 to 4 years. A highly satisfactory system can be built of concrete poles spaced about 80 feet apart and interspersed at 20-foot intervals with small posts of steel or of wood that has been treated with a preservative such as creosote. On the posts, one or more horizontal strands of No. 8 or 10 galvanized steel wire can be suspended. A means for the vines to climb to the support wire must be provided. This can be a cord such as binder twine or small wire placed at each plant position in the row. Plants will readily climb a cord tied to the support wire and pegged to the ground in the vicinity of the plant. Light stakes such as small bamboo culms or dried stems of *Sesbania exaltata* (Raf.) Cory used for supporting pole beans also can be used successfully to support dioscoreas.

A support system such as this described is quite expensive, and more economical substitutes may be available on the farm. Bamboo is available on most farms in Puerto Rico, and bamboo stakes can be used as wire supports. However, occasional concrete posts in the support system contribute to stability and permanence. If the support wires are suspended on durable structures such as creosoted wood or concrete posts, the permanence of the intermediate supporting posts becomes less critical. However, the intermediate support will require regular attention and replacement if the material used is of low durability, such as untreated bamboo poles.

MAINTAINING THE PLANTING

A field planting of *Dioscorea* species requires frequent attention, particularly in the first year when growth of the plants is rather slow and competition from weeds is severe. Small motor-driven cultivating machines have been used to eradicate weeds between rows in experimental plantings, but tractors with sufficient clearance to pass over the supporting stakes might be more practical for large fields. In all plantings some handwork with hoes or machetes has been necessary to eradicate weeds in the plant rows.

Various herbicides have been tested for weed control, but procedures are still in experimental stages and herbicide recommendations cannot yet be made. Dioscorea plants are easily injured by herbicides in their first year when they are naturally somewhat delicate and slow growing. Further studies with herbicides and plastic mulch for weed control are in progress. However, at present, careful cultivation is the only procedure that we can recommend. After the first year, vigorous vine growth produces sufficient shade to inhibit weed growth.

Although *Dioscorea* species do not appear to have any unusual and highly specific nutritional needs, a complete fertilizer is necessary for highest yields. We have routinely applied 1,000 pounds per acre per year of 9–10–5 fertilizer on soils of moderate fertility and average exchange capacity. Yields were lower when nitrogen, phosphorus, or potassium was deleted from the mixture. In general, fertilizers may be chosen for specific soil conditions according to recommendations for other vigorous root crops, such as sweetpotatoes, in that soil.

During drought, the plants may suffer and may die back to the ground. Supplemental irrigation should be profitable at such times. However, the effects of irrigation during the normally dry months have not been tested. Preliminary studies suggest that dioscoreas, particularly *D. composita*, will continue to grow during the dry season if they are irrigated.

PESTS AND DISEASES

Fortunately, sapogenin-bearing *Dioscorea* species are relatively free from attacks of pests and diseases. Nevertheless, those that do occur are sufficiently debilitating to require regular and careful inspection of the crop. Two insidious pests of all *Dioscorea* species are various species of aphids and red spiders. The aphids feed on young leaves and stems and severely stunt new growth. Young leaves and vine tips eventually die if aphids are not controlled. Older growth is seldom affected. Aphid attacks build up rapidly in the field and must be detected and treated early to avoid severe damage. Red spiders attack the underside of the leaves at the base near the petiole. Severe infestations result in necrotic areas, which are often attacked by fungi. Severe infestations may affect every leaf of the plant. In such cases new growth ceases and the plants often enter dry-season dormancy prematurely.

Tubers are frequently attacked by various chewing pests, including snails, slugs, and grubs of large beetles. Although the initial damage is seldom excessive, the tuber often rots. In areas where these pests are frequently encountered, poison baits should be used as necessary for control.

Because the foliage is dense, treatment of *Dioscorea* species with conventional dusting and spraying equipment is somewhat difficult. A high-velocity, low-volume air-blast sprayer probably would perform best for large commercial plantings. Space would have to be left in the plantings for operation of such equipment but since these machines effectively treat several rows on each side of their path, their use would not require the omission of large numbers of plant rows.

In Puerto Rico, field plantings of *Dioscorea* species have not required frequent treatment to control leaf fungi or insects. These problems are more severe on small plants, before they are transplanted from seedbeds. In the field, too, most attention is necessary when the plants are small before they become well established. At that time, they can be treated satisfactorily with small, hand-operated dusting and spraying equipment.

Various species of nematodes often attack both the tubers and the roots of *Dioscorea* species. Severe nematode injury has not been found in the relatively heavy soils of Puerto Rico. However, in light soils, root-knot nematodes may damage the plants severely.

A serious disease of *Dioscorea* species is the green-banding virus (fig. 5). This disease results in chlorotic bleaching or clearing of interveinal areas of the leaf. The alternation of dark-green and light-green tissue has suggested the name "green banding." Plants infected with the green-banding virus are seriously weakened, their top growth is restricted, and tuber growth is drastically curtailed. Plants weakened by other adverse conditions such as drought frequently die when they become infected with this virus. This is particularly true of the more susceptible species, such as *D. floribunda* and *D. friedrichsthalii*. Leaf mosaic, leaf flecking, and dwarfing of the leaf and stem are frequently associated with green banding. Mosaic and flecking symptoms appear to be occasional expression of the green-banding virus; however, dwarfing may be an expression of another disease.

Green-banding virus spreads swiftly through the field. Aphids have been shown to transmit the

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FIGURE 5.—Leaves of various *Dioscorea* species showing flecking, green banding, or mosaic induced by the green-banding virus. Healthy leaves are at left.

disease, and other pests such as leafhoppers and red spiders may also be vectors. The disease has been transmitted mechanically in controlled experiments and therefore may possibly be transmitted mechanically in the field by workers and machinery. Vegetative propagules from infected plants transmit the disease, but seeds probably do not. Seedlings from infected plants, when carefully protected against mechanical or insect transmission, have not been diseased. However, in large seedling plantings, some very small, weak seedlings have been found that may have been infected with virus. Therefore, when establishing seedbeds, it is advisable to avoid using seeds from plants that show any viruslike symptoms.

Even though the green-banding virus has been transmitted to the new host, new growth does not develop the typical symptoms of infection for several months. Presumably, plants may serve as a source of infection before symptoms develop. Thus, control measures must be based on avoiding the disease. Recommended measures include: propagation from only clean fields; complete removal and destruction of vines and tubers showing any sign of leaf mottling, flecking, or stunting (except obvious aphid damage); and planting only in areas not previously planted to *Dioscorea* species. Since a similar, if not identical, disease has been reported in the guinea yam, *D. rotundata* Poir., the sapogenin-bearing yams should not be planted with or near other yam species. No evidence has implicated the wild yams (*D. bulbifera* L. or *Rajania cordata* L.) as virus carriers, but isolation of new plantings is advisable as a precautionary measure.

Undoubtedly all cases of green-banding virus reduce tuber yields; and prompt, thorough control measures are recommended. If the disease has been identified in a field, frequent insect control treatments should be used to restrict as much as possible the spread of the disease to healthy plants.

The various *Dioscorea* species respond differently to green-banding virus infection. *D. floribunda* and *D. friedrichsthalii* are highly sensitive and react severely, and are often killed during the second season of infection. *D. composita* and *D. spiculiflora* are more resistant and may show typical symptoms but still continue to grow vigorously. Until more information about virus control is available, commercial plantings probably should not be made with *D. floribunda* alone because the risk of serious loss from virus attacks is lower in plantings of *D. composita* than in plantings of *D. floribunda.*

Fungus diseases of foliage of *Dioscorea* species have seldom been severe in continental United States or Puerto Rico. However, a *Helminthosporium* fungus severely damages leaves in Central America and occasionally damages young seedling plants in beds in Puerto Rico. Spray treatment with organic fungicides has controlled the *Helminthosporium* fungus satisfactorily.

A general wilting or dieback of vines of *Dioscorea* species has been associated in some cases with decay of the tubers, often following slug or snail damage. A few unexplained cases of wilting or dieback have been encountered, but these have not been severe.

New pests and diseases that require control measures probably will be found on *Dioscorea* species. With this new crop, to an even greater degree than with established crops, careful and frequent observation will be vital to success, and new methods may frequently be necessary as new pest control problems arise.

HARVESTING THE TUBERS

Returns cannot be expected to exceed production costs until plants grow at least 3 years in the field. Experimental plantings indicate that 3 years' growth is minimum for commercial production and that growing the plants for 4 years is preferable. During the fourth year sapogenin yields almost double those of the third, with relatively little additional maintenance or cost. Experiments in Puerto Rico have shown that the tubers continue to grow after the fourth year, but at a reduced rate.

Although tubers can be harvested at any time of the year, harvesting in the winter dry season is recommended for several reasons. Diosgenin content often drops immediately when the plants begin to grow after spring rains. Then during the growing season, new sapogenin is being deposited and delayed harvest should be profitable. During the dry season, sapogenin content is relatively stable, and harvest can proceed with less fear of losses or delay due to inclement weather. Also, if harvested tubers must be held for a period of time, they can be more easily sun-dried to a safe moisture content during the dry season.

The vines and their supports must be removed just before harvesting. With *D. floribunda* the time of vine removal is not critical. However, with *D. composita* the removal of one vine may stimulate the growth of another, and tubers should be dug soon after the vines are removed. Concrete poles and wire can be removed by hand for future use, but wooden stakes, string, and attached vines can be cut either by hand or mechanically and pushed to the sides of the field for later burning. If the field is weedy, a rotary mower or brush cutter removes vines and weeds effectively. The field should be clean before the tubers are dug.

Machinery to remove tubers from the soil has not been perfected. In a loose soil, a large moldboard plow pulled through the center of the row will excavate most of the tubers. In very heavy soils more than one furrow in each row may need to be plowed to excavate the tubers. Laborers with rakes and pitchforks must follow the plow to locate and remove tubers before the plow passes again. Once removed, tubers can be quickly brushed to remove the larger lumps of soil and thrown into waiting wheelbarrows or trucks. This harvesting system requires a great deal of hand labor. A mechanical harvesting machine built on principles of a potato or sweetpotato harvester could doubtless be devised if justified by large harvest operations. Although a machine of great strength and capacity would be necessary, it is unlikely that any special engineering innovations would be required.

Because of their smaller size, more compact form, and horizontal growth habit, tubers of *D. floribunda* can be harvested more easily and less expensively than those of *D. composita.* This lower harvest cost offsets, at least in part, the lower per acre yields of *D. floribunda.*

After harvest, the tubers should be washed to remove excess soil. The tubers can then be taken to the plant for processing. If the tubers cannot be delivered promptly to the plant, they can be sun-dried to about one-third of their green weight. The washed tubers must be cut into small chips or flakes with a hammer mill, sugarbeet chopper, or similar machine, and spread in the sun on a concrete or other clean, dry surface. These tuber chips or flakes must be turned frequently by raking, particularly in the first stages of drying, to prevent fungus growth. The material will dry in a few days if it is protected from rain and dew and turned frequently. We are assuming that mechanical drying equipment will not be available in the Tropics. Such equipment would of course make the drying procedure very simple. After proper drying, the material can be stored in sacks, but should be inspected daily and aerated, if necessary, to avoid decay. Buyers of this material will undoubtedly design standards and grades. Until such time, the principal requirement is to process the crop promptly.

Yields obtained recently in Puerto Rico are summarized in table 2. The chief factors affecting yield are species, location, soil type, method of propagation, and age at harvest.

TABLE 2.—*Yields of two sapogenin-bearing* Dioscorea *species at 4 locations in Puerto Rico*

Species and location	Soil type	Method of propagation	Age at harvest	Yield per acre: Dry matter	Yield per acre: Sapogenin		Mean sapogenin yield per acre per year
D. composita:			*Years*	*Kilograms*	*Percent*	*Kilograms*	*Kilograms*
Mayaguez	Toa silty loam	Cuttings	2	1, 513	3. 6	55	28
Do	Nipe clay	do	2	1, 710	3. 8	65	32
Do	Cialitos clay	do	2	1, 011	3. 7	37	18
Isabela	Coto loam	do	3	3, 600	6. 3	227	76
Do	do	Tuber pieces	3	2, 138	6. 5	139	46
Adjuntas	Lares clay	Cuttings	3	1, 903	6. 3	120	40
Do	do	Tuber pieces	3	1, 523	8. 3	126	42
Corozal	Estación clay	Cuttings	3	3, 047	5. 4	165	55
Do	do	Tuber pieces	3	2, 053	5. 9	121	40
Mayaguez	Catalina clay	Seeds	4	6, 526	4. 7	308	77
D. floribunda:							
Mayaguez	Toa silty loam	Cuttings	2	1, 204	4. 6	55	28
Do	Nipe clay	do	2	1, 852	4. 0	74	37
Do	Cialitos clay	do	2	453	3. 7	17	8
Isabela	Coto loam	do	3	1, 822	5. 4	98	33
Do	do	Tuber pieces	3	1, 911	5. 2	99	33
Adjuntas	Lares clay	Cuttings	3	536	6. 2	33	11
Do	do	Tuber pieces	3	968	6. 8	66	22
Corozal	Estación clay	Cuttings	3	538	5. 8	31	10
Do	do	Tuber pieces	3	936	5. 7	53	18
Mayaguez	Nipe clay	do	4	4, 195	7. 9	331	83

COSTS AND PROFITS

Production costs for all operations were estimated, and calculated profit and loss data are shown in table 3. The highest single cost in all cases was for planting the field. The most costly part of the planting operation is staking the plants. In most cases, estimated returns exceeded costs after three growing seasons, but the estimated profit margin increased considerably when the harvest was delayed until after the fourth growing season.

VARIETIES, BREEDING, AND IMPROVEMENT

Although the four *Dioscorea* species studied vary considerably, no definite varieties have been developed. Strains selected for superior performance in a particular region in their countries of origin do not necessarily yield more than nonselected strains in Puerto Rico. In replicated tests species invariably are different in fresh weight, dry weight, total sapogenin yields, and percentage of sapogenin; but differences within the species are not evident. This is probably because each strain is itself a mixture of poor to excellent plants. When individually selected plants are clonally multiplied and compared in replicated tests, differences are found. The highest yields are about 50 percent higher than the lowest. Nevertheless, calculations of the heritability of yield components are low, suggesting that environmental differences play a major role in determining yield.

Many genes probably determine yields. All the species of interest for cultivation are dioecious (fig. 6). Apparently all are tetraploids with 36 chromosomes. Since environment plays such an

TABLE 3.—*Estimated production costs for two* Dioscorea *species and profit or loss per acre from species harvested at 3 ages, as calculated from experimental data*

Species and method of propagation	Age at harvest	Costs per acre for— Propagation	Planting	Maintenance	Harvest	Total costs	Gross value	Profit or loss
D. composita:	*Years*							
Seeds	2	$33	$411	$218	$94	$756	$366	$−390
Cuttings	2	113	480	218	94	905		
Tuber pieces	2	112	432	218	94	856	246	−610
Seeds	3	33	411	334	122	900	1, 661	761
Cuttings	3	113	480	334	122	1, 049		
Tuber pieces	3	112	432	334	122	1, 000	3, 105	2, 105
Seeds	4	33	411	450	149	1, 044	2, 324	1, 280
Cuttings	4	113	480	450	149	1, 192		
Tuber pieces	4	112	432	450	149	1, 143		
D. floribunda:								
Seeds	2	33	411	218	76	738	346	−392
Cuttings	2	113	480	218	76	887	662	−225
Tuber pieces	2	112	432	218	76	838	739	−99
Seeds	3	33	411	334	96	874	690	−184
Cuttings	3	113	480	334	96	1, 023		
Tuber pieces	3	112	432	334	96	974	2, 225	1, 251
Seeds	4	33	411	450	113	1, 007	1, 193	187
Cuttings	4	113	480	450	113	1, 156		
Tuber pieces	4	112	432	450	113	1, 106	2, 138	1, 032

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FIGURE 6.—*Left,* male inflorescence; *center,* female inflorescence; *right,* fruit of *D. composita.*

important role, fixing these genes into superior varieties by conventional breeding techniques would be difficult. Selecting superior varieties would therefore be a long and tedious process, requiring many generations to complete.

A promising method to produce vigorous new varieties is to produce species hybrids. Hybridization is possible in any of the possible crosses among the four species. Some hybrids, especially those of *D. composita* with *D. floribunda* and *D. spiculiflora*, are exceptionally vigorous. This vigor is manifest in excellent vegetative propagation and tuber growth and sapogenin production. Some of these hybrids might make excellent clonal varieties.

SEED PRODUCTION

The sapogenin-bearing *Dioscorea* species are dioecious and must be cross-pollinated to set fruit. Natural cross-pollination is not effective unless the male and female flowers are within a few feet of each other and bloom at the same time. Under normal field conditions, the vines become completely entangled and set seeds freely during the second and third years. Thus, commercial plantings are sources of new seed. Seeds mature near the end of the dry season, from January to April. The pods turn brown and dehisce along the upper margin. As the pods dry, seeds must be harvested promptly or they will be lost.

Fresh seeds should be stored in airtight containers at 5 to 10° C. with a desiccant such as calcium chloride. The desiccant should not touch the seeds. Under these storage conditions seeds are viable for 3 to 5 years. However, if humidity or temperature is high, seeds may become inviable within a few months.

PROSPECTS

The sapogenin-bearing dioscoreas are a promising new crop. New crops are rare in recent history, and many problems are ordinarily encountered in attempts at domestication. The dioscoreas are no exception to this generalization. Although experimental plantings have been highly successful, experiments cannot include all the hazards that may affect commercial plantings and therefore cannot unequivocally predict that commercial plantings will be profitable.

Improving certain agronomic methods is highly important. Improvements in the methods to control weeds, support vines, and harvest tubers seem to be the most important agronomic problems. We see no reason to doubt that these improvements can be achieved fairly rapidly. The important matter of improving varieties is expected to take much more time. These problems are receiving continued attention during the critical phase between experimental work and practical application.

Although we are not in a position to analyze the economic factors that will affect future use of *Dioscorea* species, the demand for steroidal drugs seems assured. Other plant materials (stigmasterol from soybeans and glycoalkaloids from *Solanum* species) can be used for steroid synthesis; however, diosgenin is a more suitable precursor and is expected to remain the world's chief source of steroids. The principal question, therefore, seems to be whether cultivated dioscoreas can compete with the wild plant resources in southern Mexico and Asia. At this point, the prospects for cultivated dioscoreas seem favorable.

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